WEST Search History

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DATE: Wednesday, June 23, 2004

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		DB=USPT,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L7	L6 and liposome	7
☐	L6	(polyethylenimine adj3 molecular adj3 weight)	164
☐	L5	transfect$ same (polyethylenimine adj3 molecular adj3 weight)	4
☐	L4	liposome$ same (polyethylenimine adj3 molecular adj3 weight)	2
☐	L3	L2 and transfect$	15
☐	L2	peg$ceramide	17
☐	L1	liposome$ same polyethylenimine	36

END OF SEARCH HISTORY

L1: Entry 30 of 36 File: USPT Jul 22, 1997

DOCUMENT-IDENTIFIER: US 5650136 A
TITLE: Cascade polymer bound complexing compounds, their complexes and conjugates, processes for their production, and pharmaceutical agents containing them

Brief Summary Text (166):
The thus-obtained complexing ligands (as well as the complexes) can also be linked to bio- or macromolecules from which it is known that they are particularly accumulated in the organ or organ part to be examined. Such molecules are, for example, enzymes, hormones, polysaccharides, such as dextrans or starches, porphyrins, bleomycins, insulin, prostaglandins, steroid hormones, amino sugars, amino acids, peptides such as polylysine, proteins (such as, for example, immunoglobulins, monoclonal antibodies, lectins), lipids (also in the form of liposomes), and nucleotides of the DNA or RNA type. Especially to be emphasized are conjugates with albumins, such as human serum albumin, antibodies, e.g. monoclonal antibodies specific for tumor-associated antigens, or antimyosin. Instead of biological macromolecules, it is also possible to link suitable synthetic polymers, such as polyethylenimines, polyamides, polyureas, polyethers, such as polyethylene glycols, and polythioureas. The pharmaceutical agents formed therefrom are suitabe, for example, for use in tumor and infarction diagnostics, as well as tumor therapy. Monoclonal antibodies (e.g. Nature 256:495, 1975) have the advantages over polyclonal antibodies that they are specific for an antigen determinant, that they possess definite binding affinity, that they are homogeneous (thus substantially simplifying their production in pure form), and that they can be manufactured in large amounts in cell cultures. Suitable are, for example, for tumor imaging, monoclonal antibodies and/or their fragments Fab and F(ab').sub.2 which are specific, for example, for human tumors of the gastrointestinal tract, of the breast, of the liver, of the bladder, of the gonads, and of melanomas [Cancer Treatment Repts. 68:317 (1984), Bio. Sci. 34:150 (1984)] or are directed against carcinomembryonal antigen (CEA), human chorionic gonadotropin (.beta.-HCG), or other tumor-positioned antigens, such as glycoproteins [New Engl. J. Med. 298:1384 (1973), U.S. Pat. No. 4,331,647]. Suitable are, inter alia, also antimyosin, anti-insulin and antifibrin antibodies (U.S. Pat. No. 4,036,945).

L1: Entry 25 of 36 File: USPT Oct 12, 1999

DOCUMENT-IDENTIFIER: US 5965434 A
TITLE: Amphipathic PH sensitive compounds and delivery systems for delivering biologically active compounds

Brief Summary Text (15):
Various cationic metal ions and polycations have been shown to induce the fusion of negatively-charged liposomes. Polycations such as mellitin, polymixin B, polylysine and synthetic polymers such as polyethylenimine and poly(allylamine) have been shown to induce fusion at neutral pH while polymeric polycations such as polyhistidine and cetylacetyl(imidazol-4-ylmethyl)polyethylenimine (CAIPEI) induce fusion of negatively-charged liposomes at acidic pH. It is generally believed that these polymeric polycations induce fusion of negatively-charged liposomes by increasing their aggregation and presumably inducing lipid phase separation like the divalent cations. The polymeric nature of the cations is an absolute requirement for fusion since the monomeric or oligomeric cations do not induce fusion. While these polycations have been useful for studying liposome fusion they have not been used to deliver biologic substances into cells whether in culture or in the whole organism. In addition the polycations cause hemolysis and/or hemagglutination.

First Hit Fwd Refs

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L1: Entry 18 of 36 File: USPT Jul 16, 2002

DOCUMENT-IDENTIFIER: US 6420176 B1
TITLE: Composition for delivering DNA into antigen presenting cells

Detailed Description Text (21):
Once a molecule or particle is taken up into a cell via endocytosis or phagocytosis, it is contained in a protein-receptor complex called an endosome. Endosomes are intracellular acidic compartments that serve a sorting function. Phagosomes, which result from phagocytosis, are large (10.times.-20.times.) endosomes. Endosomes then fuse with lysosomes where the material is digested to smaller products such as peptides, nucleotides and sugars. In the present invention, the role of the vector is to provide the foreign gene to the cell and avoid degradation of the gene. That is, the vector must be able to break the endosome and release the gene into the intracellular fluid, cytosol, or onto the nucleus. A number of particles are known to be able to break the endosome after receptor-mediated endocytosis, including viral gene delivery particles such as adenovirus vectors, retrovirus vectors, pox-virus vectors, and SV-40 virus. Non-viral gene delivery particles include conjugates of DNA with polylysine, polyethylenimine and its derivatives, liposomes, virosomes and chemicals which increase the pH in the endosome, such as chloroquine.

Detailed Description Text (36):
The gene delivery system can include either a viral or non-viral vector. Viral gene delivery systems include recombinant virus vectors such as adenovirus vectors, retrovirus vectors, pox-virus vectors, mutant viruses (described above) and virosomes. Non-viral gene delivery systems include DNA conjugates with sugar, polylysine, polyethylenimine, polyethylenimine derivatives, and liposomes, together with their derivatives.

Detailed Description Text (37):
Non-viral gene delivery systems such as those utilizing sugars, sugar derivatives, liposomes, liposome derivatives and polyethylenimine or polyethylenimine derivatives are preferred. Of these, sugar and polyethylenimine derivatives adapted to target the mannose receptors of immune system cells are most preferred.

First Hit Fwd Refs

Generate Collection Print

L1: Entry 18 of 36 File: USPT Jul 16, 2002

US-PAT-NO: 6420176
DOCUMENT-IDENTIFIER: US 6420176 B1

TITLE: Composition for delivering DNA into antigen presenting cells

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lisziewicz; Julianna	Bethesda	MD		
Lori; Franco	Bethesda	MD		

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Genetic Immunity, LLC.	Washington	DC			02

APPL-NO: 09/ 153198 [PALM]
DATE FILED: September 15, 1998

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 60/058,933, filed Sep. 15, 1997, which is incorporated herein as if set forth in full.

INT-CL: [07] C12 N 15/09, C12 N 15/63, A61 K 48/00, A61 K 35/00

US-CL-ISSUED: 435/455
US-CL-CURRENT: 435/455

FIELD-OF-SEARCH: 536/23.1, 424/93.1, 424/208.1, 424/184.1, 514/44, 435/320.1, 435/325, 435/235.1, 435/455

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	6013240	January 2000	Behr	

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Condon, et al., DNA-based immunization by in vivo transduction of dendritic cells, Nature Medicine 2:10 1122-1128 (1996).
Reiser, et al., Transduction of nondividing cells using pseudotyped defective high-titer HIV type 1 particles, Proc Natl Acad Sci USA 93: 15266-15271 (1996).

ART-UNIT: 1633

PRIMARY-EXAMINER: Wilson; Michael C.

ATTY-AGENT-FIRM: Looper; Valerie E.

ABSTRACT:

A molecular delivery complex specific to antigen-presenting cells is formed from a a non-viral gene delivery system complexed with foreign genetic material. The complex then enters the targeted cells through a specific receptor and overcomes the degradation mechanism, so that functional uptake of the foreign genetic

material, or transduction, of the cell, results in gene expression. The invention also includes a method for genetic immunization without a needle.

14 Claims, 7 Drawing figures

L1: Entry 9 of 36 File: USPT Jul 1, 2003

DOCUMENT-IDENTIFIER: US 6586524 B2
TITLE: Cellular targeting poly(ethylene glycol)-grafted polymeric gene carrier

Brief Summary Text (6):
On the other hand, non-viral gene delivery systems such as cationic liposomes or synthetic cationic polymers, e.g.poly-L-lysine (PLL) and polyamines, are being widely sought as alternatives. M. A. Wolfert et al., Characterization of Vectors for Gene Therapy Formed by Self-Assembly of DNA with Synthetic Block Co-Polymers. 7 Hum. Gene. Ther., 2123-2133 (1996); A V Kabanov & V A Kabanov DNA Complexes with Polycations for the Delivery of Genetic Materials into Cells. 6 Bioconj. Chem., 7-20 (1995). There are several advantages to the use of non-viral based gene therapies including their relative safety and low cost of manufacture. The major limitation of plasmid DNA(pDNA)-based approaches has been that both the efficiency of gene delivery to important somatic targets (i.e., liver, lung and tumors) and in vivo gene expression levels are lower using non-viral approaches than those using viral vectors. Polyethylenimine (PEI), one of the most commonly used cationic polymers, mediates a high degree of transfection due to the release of pDNA from the endosome to the cytosol. O.Boussif, F. Lezoualc'h, M. A. Zanta, M. D. Mergny, D. Scherman, B. Demeneix, J. P. Behr, A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: Polyethylenimine. Proc. Natl. Acad. Sci. USA. 92 (1995) 7297-7301. Although PEI condenses pDNA into complexes of less than 50 nm in salt-free buffer, these complexes aggregate immediately under physiological conditions such as in salt or bovine serum albumin.

L3: Entry 14 of 15 File: USPT Jan 6, 1998

DOCUMENT-IDENTIFIER: US 5705385 A
TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

Brief Summary Text (7):
One method of introducing nucleic acids into a cell is mechanically, using direct microinjection. However this method is only practical for transfecting eukaryotic germline cells for the production of transgenic systems. To be effective in treating a disease, a nucleic acid-based therapy must enter many cells.

Brief Summary Text (10):
Lipid-based vectors have also been used in gene transfer and have been formulated in one of two ways. In one method, the nucleic acid is introduced into preformed liposomes made of mixture of cationic lipids and neutral lipids. The complexes thus formed have undefined and complicated structures and the transfection efficiency is severely reduced by the presence of serum. Preformed liposomes are commercially available as LIPOFECTIN.RTM. and LIPOFECTAMINE.RTM.. The second method involves the formation of DNA complexes with mono- or poly-cationic lipids without the presence of a neutral lipid. These complexes are prepared in the presence of ethanol and are not stable in water. Additionally, these complexes are adversely affected by serum (see, Behr, Acc. Chem. Res. 26:274-78 (1993)). An example of a commercially available poly-cationic lipid is TRANSFECTAM.RTM..

Brief Summary Text (12):
Ideally, a delivery vehicle for nucleic acid will be small enough (<200 nm) and stable enough in circulation to distribute from local injection sites or following intravenous injection. The composition will have the maximum amount of nucleic acid per particle and will be homogenous and reproducible. The composition should also maintain the nucleic acid in a configuration which is protected from degradation prior to nuclear delivery and should efficiently transfect the target cells.

Brief Summary Text (25):
It is a further aspect of the invention to provide in vitro and in vivo methods for treatment of diseases which involve the overproduction or underproduction of particular proteins. In these methods, a nucleic acid encoding a desired protein or blocking the production of an undesired protein, is formulated through a hydrophobic intermediate into a lipid-nucleic acid particle, and the particles are administered to patients requiring such treatment. Alternatively, cells are removed from a patient, transfected with the lipid-nucleic acid particles described herein, and reinjected into the patient.

Drawing Description Text (14):
FIG. 13 provides the results of CHO cell transfection using .beta.-gal plasmid/DODAC/ESM as assayed by .beta.-gal enzyme activity.

Detailed Description Text (18):
The term "cationic lipid" refers to any of a number of lipid species which carry a net positive charge at physiological pH. Such lipids include, but are not limited to, DODAC, DOTMA, DDAB, DOTAP, DC-Chol and DMRIE. Additionally, a number of commercial preparations of cationic lipids are available which can be used in the

present invention. These include, for example, LIPOFECTIN.RTM. (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, N.Y., USA); LIPOFECTAMINE.RTM. (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM.RTM. (commercially available cationic lipids comprising DOGS in ethanol from Promega Corp., Madison, Wis., USA).

Detailed Description Text (19):
The term "transfection" as used herein, refers to the introduction of polyanionic materials, particularly nucleic acids, into cells. The term "lipofection" refers to the introduction of such materials using liposome complexes. The polyanionic materials can be in the form of DNA or RNA which is linked to expression vectors to facilitate gene expression after entry into the cell. Thus the polyanionic material used in the present invention is meant to include DNA having coding sequences for structural proteins, receptors and hormones, as well as transcriptional and translational regulatory elements (i.e., promoters, enhancers, terminators and signal sequences) and vector sequences. Methods of incorporating particular nucleic acids into expression vectors are well known to those of skill in the art, but are described in detail in, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd ed.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989) or Current Protocols in Molecular Biology, F. Ausubel et al., ed. Greene Publishing and Wiley-Interscience, New York (1987), both of which are incorporated herein by reference.

Detailed Description Text (23):
The present invention provides lipid-nucleic acid particles produced via novel, hydrophobic nucleic acid-lipid intermediate complexes. The complexes are charge-neutralized. Manipulation of these complexes in either detergent-based or organic solvent-based systems can lead to particle formation in which the nucleic acid is protected and in which particle components can be altered to improve transfection efficiencies in vitro and in vivo. Gene delivery in vitro can be improved, for example, through incorporation of a lipid, such as biotinylated phospholipids, that can facilitate targeting via avidin linked monoclonal antibodies. In vivo pharmacokinetic properties can be improved for example, by i) incorporation of cholesterol, ii) control of particle size, iii) elimination of surface charge and/or iv) incorporation of lipids (e.g., PEG-modified lipids) that reduce protein binding and reticuloendothelial cell uptake.

Detailed Description Text (27):
In one aspect, the present invention provides novel, lipid-nucleic acid complexes consisting essentially of cationic lipids and nucleic acids. These complexes can be distinguished from other complexes by several features. In particular, these complexes have a hydrophobic character (being soluble in organic solvents) and are charge-neutralized. Additionally, the nucleic acid portion of the complexes exists in an uncondensed form. These complexes can be used in the preparation of the lipid-nucleic acid particles which are described below and which are themselves useful for transfecting cells in vitro or in vivo.

Detailed Description Text (28):
The complexes consist essentially of cationic lipids and nucleic acids. The cationic lipids can be any of a number of lipid species which carry a net positive charge at physiological pH, including, for example DODAC, DOTMA, DDAB, DOTAP, DOSPA, DC-Chol, DOGS and DMRIE. Additionally, a number of commercial preparations of cationic lipids are available which can be used in the present invention. These include, for example, LIPOFECTIN.RTM.; LIPOFECTAMINE.RTM. and TRANSFECTAM.RTM..

Detailed Description Text (46):
The nucleic acid-lipid mixture thus formed is contacted with cationic lipids to neutralize a portion of the negative charge which is associated with the nucleic acids (or other polyanionic materials) present. The amount of cationic lipids used

will typically be sufficient to neutralize at least 50% of the negative charge of the nucleic acid. Preferably, the negative charge will be at least 70% neutralized, more preferably at least 90% neutralized. Cationic lipids which are useful in the present invention, include, for example, DODAC, DOTMA, DDAB, DOTAP, DC-Chol and DMRIE. These lipids and related analogs have been described in co-pending U.S. Ser. No. 08/316,399; U.S. Pat. Nos. 5,208,036, 5,264,618, 5,279,833 and 5,283,185, the disclosures of which are incorporated herein by reference. Additionally, a number of commercial preparations of cationic lipids are available and can be used in the present invention. These include, for example, LIPOFECTIN.RTM. (commercially available cationic liposomes comprising DOTMA and DOPE, from GIBCO/BRL, Grand Island, N.Y., USA); LIPOFECTAMINE.RTM. (commercially available cationic liposomes comprising DOSPA and DOPE, from GIBCO/BRL); and TRANSFECTAM.RTM. (commercially available cationic lipids comprising DOGS in ethanol from Promega Corp., Madison, Wis., USA).

Detailed Description Text (50):
In other embodiments, the methods will further comprise adding nonlipid polycations which are useful to effect the transfection of cells using the present compositions. Examples of suitable nonlipid polycations include, hexadimethrine bromide (sold under the brandname POLYBRENE.RTM., from Aldrich Chemical Co., Milwaukee, Wis., USA) or other salts of hexadimethrine. Other suitable polycations include, for example, salts of poly-L-ornithine, poly-L-arginine, poly-L-lysine, poly-D-lysine, polyallylamine and polyethyleneimine. Addition of these salts is preferably after the particles have been formed.

Detailed Description Text (60):
In yet another aspect, the present invention provides lipid-nucleic acid particles which are prepared by the methods described above. In these embodiments, the lipid-nucleic acid particles are either net charge neutral or carry an overall charge which provides the particles with greater gene transfection activity. Preferably, the nucleic acid component of the particles is a nucleic acid which encodes a desired protein or blocks the production of an undesired protein. In particularly preferred embodiments, the nucleic acid is a plasmid, the non-cationic lipid is egg sphingomyelin and the cationic lipid is DODAC.

Detailed Description Text (61):
As noted above, the lipid-nucleic acid particles are useful for the transfection of cells, either in vitro or in vivo. Accordingly, the present invention provides, in yet another aspect, a method for introducing a nucleic acid into a cell, comprising;

Detailed Description Text (69):
As noted above, it is often desirable to include polyethylene glycol (PEG)-modified phospholipids, PEG-ceramide, or ganglioside G.sub.M1 -modified lipids to the particles. Addition of such components prevents particle aggregation and provides a means for increasing circulation lifetime and increasing the delivery of the lipid-nucleic acid particles to the target tissues. Typically, the concentration of the PEG-modified phospholipids, PEG-ceramide or G.sub.M1 -modified lipids in the particle will be about 1-15%.

Detailed Description Text (75):
Following formation of the lipid-nucleic acid particles, the particles can be contacted with the cells to be transfected. The particles can be adsorbed to almost any cell type. Once adsorbed, the particles can either be endocytosed by a portion of the cells, exchange lipids with cell membranes, or fuse with the cells. Transfer or incorporation of the nucleic acid portion of the particle can take place via any one of these pathways. In particular, when fusion takes place, the particle membrane is integrated into the cell membrane and the contents of the particle combine with the intracellular fluid. Contact between the cells and the lipid-nucleic acid particles, when carried out in vitro, will take place in a

biologically compatible medium. The concentration of particles can vary widely depending on the particular application, but is generally between about 1 .mu.mol and about 10 mmol. Treatment of the cells with the lipid-nucleic acid particles will generally be carried out at physiological temperatures (about 37.degree. C.) for periods of time of from about 1 to 48 hours, preferably of from about 2 to 4 hours. For in vitro applications, the delivery of nucleic acids can be to any cell grown in culture, whether of plant or animal origin, vertebrate or invertebrate, and of any tissue or type. In preferred embodiments, the cells will be animal cells, more preferably mammalian cells, and most preferably human cells.

Detailed Description Text (77):
Typical applications include using well known transfection procedures to provide intracellular delivery of DNA or mRNA sequences which code for therapeutically useful polypeptides. However, the compositions can also be used for the delivery of the expressed gene product or protein itself. In this manner, therapy is provided for genetic diseases by supplying deficient or absent gene products (i.e., for Duchenne's dystrophy, see Kunkel, et al., Brit. Med. Bull. 45(3):630-643 (1989), and for cystic fibrosis, see Goodfellow, Nature 341:102-103 (1989)). Other uses for the compositions of the present invention include introduction of antisense oligonucleotides in cells (see, Bennett, et al., Mol. Pharm. 41:1023-1033 (1992)).

Detailed Description Text (78):
Alternatively, the compositions of the present invention can also be used for the transfection of cells in vivo, using methods which are known to those of skill in the art. In particular, Zhu, et al., Science 261:209-211 (1993), incorporated herein by reference, describes the intravenous delivery of cytomegalovirus (CMV)-chloramphenicol acetyltransferase (CAT) expression plasmid using DOTMA-DOPE complexes. Hyde, et al., Nature 362:250-256 (1993), incorporated herein by reference, describes the delivery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene to epithelia of the airway and to alveoli in the lung of mice, using liposomes. Brigham, et at., Am. J. Med. Sci. 298:278-281 (1989), incorporated herein by reference, describes the in vivo transfection of lungs of mice with a functioning prokaryotic gene encoding the intracellular enzyme, chloramphenicol acetyltransferase (CAT).

Detailed Description Text (84):
In the examples below, Examples 1-7 illustrate the formation and characterization of charge-neutralized lipid-nucleic acid intermediate complexes, in which the nucleic acid adopts hydrophobic character. In each of these examples, the term "DNA" or "plasmid" refers to the plasmid pCMV.beta.. Examples 8 and 9 illustrate the preparation and characterization of lipid-nucleic acid particles which are suitable for transfection of cells. Examples 10-12 illustrate the serum stability and transfecting ability of these lipid-nucleic acid particles.

Detailed Description Text (86):
Transfecting agents Lipofectin and Lipofectamine were purchased from Gibco/BRL (Grand Island, N.Y., USA). Transfectam Reagent was purchased from Promega Corp. (Madison, Wis., USA). The monocationic lipid DDAB, calcium chloride, L-lysine (free base), poly L-lysine hydrobromide (Avg. MW 52,000), n-octyl .beta.-D-glucopyranoside (OGP) and DNase I were obtained from Sigma Chemical Company (St. Louis, Mo., USA). TO-PRO-1 (thiazole orange monomer) was obtained from Molecular Probes Inc., Eugene, Oreg., USA. The plasmid pCMV.beta. (GenBank accession #U02451) encoding E. coli .beta.-galactosidase (.beta.-gal), a 7.2 kb plasmid DNA reporter gene, was obtained from Clontech Laboratories, Palo Alto, Calif., USA.

Detailed Description Text (108):
LIPOFECTAMINE.RTM. (DOSPA:DOPE, 75:25 mol ratio), and TRANSFECTAM.RTM. (100% DOGS) were added to DNA (10 .mu.g) as preformed liposomes, as described in Example 2. The liposomes contain headgroups derived from spermine and exhibit positive charges of 5 and 4, respectively at pH<7. As expected, significantly lower amounts of these

lipids (calculated on the basis of moles) are required to mediate DNA partitioning into the organic phase (see FIG. 4). Complete partitioning of the DNA into the organic phase was achieved after addition of approximately 10 nmoles DOSPA and DOGS.

Detailed Description Text (133):
A lipid-nucleic acid particle formulation was prepared according to the procedure described in Example 8. Portions of the formulation (using either ESM or DOPE as the neutral lipid) were combined with PBS (140 mM NaCl, 10 mM Na.sub.2 HPO.sub.4) or serum-containing medium and incubated for two hours at 37.degree. C. The resulting complexes were isolated and examined for any changes in QELS size results or transfection efficiency. No difference was found for any of the formulations, indicating that the complexes were not disrupted by either sodium or serum components. One portion which was incubated with PBS for 10 days still showed very good transfection efficiency.

Detailed Description Text (138):
This example illustrates the in vitro transfection of CHO or B16 cell lines using lipid-nucleic acid particles prepared by the method of Example 8.

Detailed Description Text (139):
In vitro transfection was performed using a 96-well cell culture plate (Costar, Cambridge, Mass., USA) containing 50% confluent growth of either Chinese Hamster Ovary (CHO) or murine melanoma (B16) cell lines. Appropriate amounts (about 6-50 .mu.L) of the lipid-nucleic acid particle formulation (10 .mu.g DNA/mL) were premixed with medium containing 10% serum to a final volume of 150 .mu.L. The medium surrounding the cells was removed using a needle syringe and replaced with the lipid-nucleic acid particles in 10% serum-containing medium. The cells and complex were incubated for a further 48 hours at 37.degree. C. The transfection efficiency was evaluated using .beta.-gal stain or an enzyme activity assay. Results are presented in FIG. 13.

Detailed Description Text (140):
The transfection study showed excellent transfection efficiency with ESM-containing complexes and with DOPE-containing complexes (not shown). A cationic lipid to DNA charge ratio of 3:1 to 4:1 gave the best in vitro transfection results.

Other Reference Publication (1):
Hawley-Nelson, et al. Focus 15(3):73 (1993) Lipofectamine.TM.Reagent: A New,Higher Efficiency Polycationic Liposome Transfection Reagent.

L3: Entry 13 of 15 File: USPT Oct 13, 1998

DOCUMENT-IDENTIFIER: US 5820873 A
**** See image for Certificate of Correction ****
TITLE: Polyethylene glycol modified ceramide lipids and liposome uses thereof

Brief Summary Text (3):
The present invention relates to novel polyethylene glycol (PEG) derivatized lipids, their method of preparation and their use in liposomes or other lipid-based carriers. More specifically, the present invention includes PEG-Ceramide lipids and their inclusion in liposomes for use in drug delivery.

Brief Summary Text (22):
In another aspect, the present invention includes liposomes or other lipid-based carriers including the above-described PEG-Ceramide lipids. Preferred liposome compositions include the preferred lipids described above. In construction of the liposomes, various mixtures of the described PEG-Ceramide lipids can be used in combination and in conjunction with other lipid types, such as DOPE and DODAC, as well as DSPC, SM, Chol and the like, with DOPE and DODAC preferred. Typically, the PEG-Ceramide will comprise about 5 to about 30 mol % of the final liposome construction, but can comprise about 0.0 to about 60 mol % or about 0.5 to about 5 mol %. More preferred lipid compositions are those wherein a drug or a biological agent is encapsulated within the liposome. The invention also includes lipid complexes whereby the PEG-Ceramide lipid comprises about 0.01 to about 90 mol % of the complex.

Brief Summary Text (23):
In still another aspect, the present invention includes methods for delivering therapeutic agents such as drugs and vaccines to a patient in need thereof comprising administering to the patient a therapeutically effective amount of such therapeutic agent in a liposome or a lipid-based carrier of the invention. Also provided are kits for preparing labeled liposomes, containing the PEG-Ceramide lipids, and pharmaceutical formulations containing liposomes.

Drawing Description Text (7):
FIG. 6 graphically shows the effect of increasing concentrations of PEG-Ceramide (C20) on biodistribution of liposomes in the blood and liver. .sup.3 H-labeled liposomes composed of DOPE (dioleoylphosphatidylethanolamine), 15 mol % DODAC (N,N-dioleoyl-N,N-dimethylammonium chloride) and the indicated concentrations of PEG-Ceramide (C20) were injected i.v. into mice. Biodistribution was examined at 1 hour after injection, and the data were expressed as a percentage of the injected dose in the blood (upper panel) and liver (lower panel) with SD (standard deviation) (n=3).

Drawing Description Text (8):
FIG. 7 graphically illustrates the effect of increasing concentrations of DODAC on the biodistribution of liposomes in the blood. .sup.3 H-labeled liposomes composed of DOPE, 10 (open squares) or 30 (open triangles) mol % PEG-Ceramide (C20), and the indicated concentration of DODAC were injected i.v. into mice. Biodistribution was examined at 1 hour after injection, and the data were expressed as a percentage of the injected dose in the blood with SD (n=3).

Drawing Description Text (9):
FIG. 8 graphically shows the liposome levels in the blood and liver at different times after injection. .sup.3 H-labeled liposomes composed of DOPE/DODAC (85:15 mol/mol) (open circles with 0% PEG-Ceramide (C20)), DOPE/DODAC/PEG-Ceramide (C20) (75:15:10 mol/mol/mol) (open squares with 10% PEG-Ceramide (C20)), and DOPE/DODAC/PEG-Ceramide (C20) (55:15:30 mol/mol/mol) (open triangles with 30% PEG-Ceramide (C20)) were injected i.v. into mice. Biodistribution was examined at indicated times, and the data were expressed as a percentage of the injected dose in the blood (upper panel) and in the liver (lower panel) with SD (n=3).

Detailed Description Text (2):
The PEG-modified ceramide lipids of Formula I enhance the properties of liposomes by increasing the circulation longevity or lifetime of the liposome; preventing aggregation of the liposomes during covalent protein coupling, such as for targeting; preventing aggregation of liposomes incorporating targeting moieties or drugs, such as antibodies, and DNA; promoting drug retention within the liposome; and/or increasing bilayer or other stability of the liposome when low pH is required for encapsulation of the bioactive agents. These PEG-Ceramide lipids also reduce leakage due to hydrolysis of the fatty acyl chains of the liposome bilayer and are more stable than other lipid forms.

Detailed Description Text (19):
A general sequence of reactions for forming the compound of the invention is illustrated below in Reaction Scheme I. As shown therein, ceramide derivative 1 is reacted with the PEG derivative PEG-Y.sup.1 -alk-RG. R.sup.1 -R.sup.4 and Y.sup.1 have their meanings as defined above. RG is a group which reacts with X.sup.2 to form the desired linkage Y.sup.2 between PEG and the ceramide derivative (i.e., --Y.sup.1 -alk-Y.sup.2 --). Thus, it will be appreciated that the identities of RG and X.sup.2 will be complementary to each other and defined in such a way as to provide the desired linkage. For example, where RG is a nucleophilic center, such as --SH, --OH, or --NH.sub.2, X.sup.2 may be oxygen derivatized to form a good leaving group, such as --OTs where Ts represents the tosyl group, or halogen. Conversely, X.sup.2 may be a nucleophilic center, e.g., --SH, --OH, or --NH.sub.2, and RG a group which is reactive toward nucleophilic attack, e.g., carboxyl activated with dicyclohexylcarbodiimide (DCC) or acyl chloride (--COCl). By suitable choice of RG and X.sup.2, the desired amido, amine, ether, ester, thioether, carboxyl, carbamate, carbonyl, carbonate, urea or phosphoro coupling between the linker and the ceramide may be obtained. Finally any protecting groups, e.g., R.sup.8, remaining on the intermediate 2 are converted to form the desired PEG-Ceramide derivative 3. ##STR2##

Detailed Description Text (20):
An exemplary synthesis of the PEG-Ceramide lipids of the invention wherein Y.sup.1 and Y.sup.2 are carboxyl is illustrated below in Reaction Scheme II. To eliminate the potential problem of crosslinkage formation, PEG is capped at one end by an unreactive group such as methoxy or ethoxy. The second hydroxy group at the other terminal of the PEG molecule is either activated with a suitable reagent such as cyanuric acid, 1,1'-carbonyldiimidazole (CDI) or tresyl halide. Alternatively the terminal hydroxyl group may first be converted to a derivative that can be readily reacted with ceramide in the presence of appropriate condensation reagents, such as the succinate or amine. In other alternative methods, the hydroxy groups on ceramide can be selectively activated for conjugation with PEG, or the two compounds can be linked in a concerted coupling reaction by established coupling procedures.

Detailed Description Text (23):
Once both of the hydroxyl groups are protected, the C.sub.1 --OH protecting group is removed under conditions which do not affect the protecting group at the C.sub.3 alcohol. The free hydroxyl function is then reacted with the PEG derivative Me(PEG)OC(O)CH.sub.2 CH.sub.2 CO.sub.2 H with dicyclohexylcarbodiimide (DCC) and 4-N,N'-

dimethylaminopyridine (DMAP) to form the desired PEG-Ceramide derivative.

Detailed Description Text (66):
Since the present liposomes made from PEG-Ceramide lipids are less susceptible to hydrolysis, they have a prolonged half-life resulting in prolonged circulation. Additionally, the liposome pharmaceutical composition can include lipid-protective agents that protect the liposomes against free-radical and lipid-peroxidative damage upon storage. Such protective agents include alpha-tocopherol and water-soluble, iron-specific chelators, such as ferrioxamine.

Detailed Description Text (69):
Cationic lipids have been used in the transfection of cells in vitro and in vivo (Wang C-Y, Huang L. pH-sensitive immunoliposomes mediate target cell-specific delivery and controlled expression of a foreign gene in mouse. PROC. NATL. ACAD. SCI USA, 1987; 84:7851-7855 and Hyde S. C., Gill D. R. Higgins C. F., et al. Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy. NATURE. 1993;362:250-255.) The efficiency of this transfection has often been less than desired, for various reasons. One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.

Detailed Description Text (70):
Cationic lipids useful in producing lipid-based carriers for gene and oligonucleotide delivery are LIPOFECTIN (U.S. Pat. Nos. 4,897,355; 4,946,787; and 5,208,036 by Eppstein et al.) and LIPOFECTACE (U.S. Pat. No. 5,279,883 by Rose). Both agents, as well as other transfecting cationic lipids, are available from Life Technologies, Inc. in Gaithersburg, Md.

Detailed Description Text (101):
Various PEG-Ceramide Acyl Chain Lengths and Effects on Retention Time Methods

Detailed Description Text (109):
The results presented in FIG. 3 (Lipid T.sub.1/2) show that the half-life of SM/cholesterol liposomes containing 5 mol % PEG.sub.2000 -DSPE is approximately two-fold greater than SM/cholesterol liposomes containing PEG.sub.2000 -ceramides (PEG-Ceramide), regardless of the chain length. For the PEG-Ceramides, there was no significant influence of fatty acyl chain length on circulation longevity in these vincristine-loaded liposomes.

Detailed Description Text (110):
The results presented in FIG. 4 (vincristine/lipid T.sub.1/2) indicate that there is a significant influence of acyl chain length on vincristine retention in the liposomes during circulation in the plasma. Specifically, C20 PEG-Ceramide was retained significantly better than both shorter (C8, C14, egg-CER and C24) chain lengths of PEG-Ceramide and also better than PEG-DSPE. The C20 chain lengths of PEG-Ceramide had half-life values for the vincristine/lipid ratio of 28-30 hours; about twice as long as those observed for the poorest vincristine retaining formulations at 15 hours (C8 PEG-Ceramide and PEG-DSPE).

Detailed Description Text (111):
The combined result of lipid circulation longevity and drug retention within these liposomes is the circulation half-lives of vincristine (see FIG. 5). Amongst the PEG-Ceramides, the C20 chain length resulted in the greatest circulation lifetime for the vincristine (9.5-10.5 hours T1/2 vs. 7-9 hours for the C8, C14, C24 and egg-CER chain lengths). In the samples containing PEG-DSPE, the combined influence of longer liposome circulation lifetime (FIG. 3) contrasted with poor vincristine retention (FIG. 4), resulted in overall drug half-life very similar to the C20 PEG-Ceramide.

Detailed Description Text (114):

The ability of amphipathic polyethyleneglycol (PEG) derivatives to stabilize fusogenic liposomes containing a cationic lipid in vivo were examined in this study. A freeze-fracture electron microscope analysis of liposomes composed of dioleoylphosphatidylethanolamine (DOPE) and N,N-dioleoyl-N,N-dimethylammonium chloride (DODAC) showed that inclusion of amphipatic PEG derivatives, PEG-DSPE and PEG-Ceramide (PEG-Ceramide) effectively prevented liposome aggregation in the presence of mouse serum. Biodistribution of fusogenic liposomes composed of DOPE and DODAC, additionally containing an amphipathic polyethyleneglycol (PEG) derivative, were then examined in mice using .sup.3 H-labelled cholesterylhexadecylether as a lipid marker. Amphipathic PEG derivatives included PEG-DSPE and various PEG-Ceramide (PEG-Cer) with different acyl chain length ranging from C8 to C24. DOPE/DODAC liposomes (85:15, mol/mol) were shown to be cleared rapidly from the blood and accumulate exclusively in the liver. Inclusion of amphipathic PEG derivatives at 5.0 mol % of the lipid mixture resulted in increased liposome levels remaining in the blood and concomitantly decreased accumulation in the liver. Among various amphipathic PEG derivatives, PEG-DSPE shows the highest activity in prolonging the circulation time of DOPE/DODAC liposomes. The activity of PEG-Ceramide is directly proportional to the acyl chain length: the longer the acyl chain, the higher the activity. The activity of PEG-Ceramide (C20) exhibiting the optimal acyl chain length depends on its concentration of the lipid mixture with the maximal circulation time obtained at 30 mol % of the lipid mixture. While inclusion of amphipathic PEG derivatives in the lipid composition generally results in increased circulation time of DOPE/DODAC liposomes, the presence of a cationic lipid, DODAC, appeared to promote their rapid clearance from the blood.

Detailed Description Text (126):
DOPE/DODAC liposomes with or without amphipathic PEG derivatives were prepared to include .sup.3 H-labelled cholesterol hexadecylether as a lipid marker, and their biodistribution was examined in mice at 1 hour after injection. Liposomes tested in this study were composed of DOPE/DODAC (85:15, mol/mol), DOPE/DODAC/PEG-Ceramide (80:15:5, mol/mol), and DOPE/DODAC/PEG-DSPE (80:15:5, mol/mol). To also examine the effect of the hydrophobic anchor on biodistribution of liposomes, various PEG-Ceramide derivatives with different acyl chain lengths were used. These liposomal formulations had similar average diameters, ranging from 89 to 103 nm. Table II below shows levels of liposomes in the blood, spleen, liver, lung, heart, and kidney, together with respective blood/liver ratios. DOPE/DODAC liposomes were shown to be cleared rapidly from the blood and accumulate exclusively in the liver with the blood/liver ratio of approximately 0.01. Inclusion of amphipathic PEG derivatives at 5.0 mol % in the lipid composition resulted in their increased blood levels and accordingly decreased liver accumulation to different degrees. DOPE/DODAC/PEG-DSPE liposomes showed the highest blood level (about 59%) and the lowest liver accumulation (about 35%) with the blood/liver ratio of approximately 1.7 at 1 hour after injection. Among various PEG-Ceramide derivatives with different acyl chain lengths, PEG-Ceramide (C20)-containing liposomes showed the highest blood level (about 30%) with the blood/liver ratio of approximately 0.58, while PEG-Ceramide (C8)-containing liposomes showed a lower blood level (about 6%) with the blood/liver ratio of approximately 0.1. It appeared that, among different PEG-Ceramide derivatives, the activity in increasing the blood level of liposomes is directly proportional to the acyl chain length of ceramide; the longer the acyl chain length, the greater the activity. It also appeared that the optimal derivative for increasing the blood level of liposomes is PEG-Ceramide (C20).

Detailed Description Text (128):
The effect of increasing concentrations of PEG-Ceramide (C20) in the lipid composition on biodistribution of DOPE/DODAC liposomes was examined. PEG-Ceramide (C20) was included in DOPE/DODAC liposomes at increasing concentrations (0-30 mol %) in the lipid composition, while the concentration of DODAC was kept at 15 mol % of the lipid mixture. Liposomes were prepared by the extrusion method and had similar average diameters ranging from 102 nm to 114 nm. Liposomes were injected

i.v. into mice, and biodistribution was examined at 1 hour after injections. FIG. 6 shows the liposome level in the blood and liver at 1 hour after injections as a function of the PEG-Ceramide (C20) concentration. Clearly, increasing the concentration of PEG-Ceramide in the lipid composition resulted in progressive increase in liposome levels in the blood, accompanied by decreased accumulation in the liver. The highest blood level (about 84% at 1 hour after injection) was obtained for DOPE/DODAC/PEG-Ceramide (C20) (55:15:30, mol/mol) showing the blood/liver ratio of about 6.5.

Detailed Description Text (129):
The effect of increasing concentrations of DODAC on the biodistribution of DOPE/DODAC liposomes also was examined. DOPE/DODAC liposomes containing either 10 mol % or 30 mol % PEG-Ceramide (C20) and various concentrations (15, 30, 50 mol %) were prepared by the extrusion method and had similar average diameters ranging from 103 to 114 nm. Biodistribution was examined at 1 hour after injections, and expressed as percentages of liposomes in the blood as a function of the DODAC concentration (FIG. 7). As shown in FIG. 7, increasing DODAC concentrations in the lipid composition resulted in decreased levels in the blood for both liposomal formulations. Thus, the presence of a cationic lipid, DODAC, in the lipid composition results in rapid clearance from the blood. Also, shown in FIG. 7 is that such a DODAC effect can be reversed by increasing the concentration of PEG-Ceramide (C20) in the lipid composition.

Detailed Description Text (130):
FIG. 8 shows time-dependent clearances of DOPE/DODAC liposomes with or without PEG-Ceramide from the blood. Only a small fraction of injected DOPE/DODAC liposomes remained in the blood, while increasing the concentration of PEG-Ceramide (C20) in the lipid composition resulted in prolonged circulation times in the blood. Estimated half-lives in the .alpha.-phase for DOPE/DODAC/PEG-Ceramide (C20) (75:15:10, mol/mol) and DOPE/DODAC/PEG-Ceramide (C20) (55:15:30, mol/mol) were <1 hour and 5 hours, respectively.

Detailed Description Text (132):
The above studies indicate that there are several levels at which biodistribution of fusogenic liposomes containing a cationic lipid can be controlled by inclusion of amphipathic PEG derivatives. Data in Table II shows that the hydrophobic anchor of amphipathic PEG derivatives has an important role in determining biodistribution of DOPE/DODAC liposomes. Studies using various PEG-Ceramide derivatives with different acyl chain lengths showed that the longer the acyl chain length of PEG-Ceramide, the greater the activity in prolonging the circulation time of DOPE/DODAC liposomes. These results are consistent with the rate at which amphipathic PEG derivatives dissociate from the liposome membrane is directly proportional to the size of the hydrophobic anchor. Accordingly, PEG-Ceramide derivatives with a longer acyl chain can have stronger interactions with other acyl chains in the liposome membrane and exhibit a reduced rate of dissociation from the liposome membrane, resulting in stabilization of DOPE/DODAC liposomes for a prolonged period of time and thus their prolonged circulation time in the blood.

Detailed Description Text (133):
In addition to the hydrophobic anchor of amphipathic PEG derivatives, the concentration of amphipathic PEG derivatives in the lipid membrane can also be used to control in vivo behavior of DOPE/DODAC liposomes. Data in FIG. 6 show that increasing the concentration of PEG-Ceramide (C20) in the lipid composition resulted in increased liposome levels in the blood. The optimal concentration of PEG-Ceramide (C20) in the lipid composition was found to be 30 mol % of the lipid mixture. It appeared that the circulation time of DOPE/DODAC/PEG-Ceramide (C20) liposomes is determined by the relative concentrations of two lipid compositions, DOPE and PEG-Ceramide, exhibiting opposite effects on liposome biodistribution. While amphipathic PEG derivatives show the activity in prolonging the circulation time of liposomes in -the blood, a cationic lipid, DODAC, shows the activity to

facilitate liposome clearance from the blood. Thus, for the maximal circulation time in the blood, an appropriate concentration of amphipathic PEG derivatives and a minimal concentration of DODAC should be used. It should be noted, however, that an optimal liposome formulation for the prolonged circulation time in the blood is not necessarily the one suitable for an intended application in delivery of certain therapeutic agents. Both pharmacokinetic and pharmacodynamic aspects of fusogenic liposomes should be examined for different applications using different therapeutic agents.

L3: Entry 12 of 15 File: USPT Mar 23, 1999

DOCUMENT-IDENTIFIER: US 5885613 A
**** See image for Certificate of Correction ****
TITLE: Bilayer stabilizing components and their use in forming programmable fusogenic liposomes

Drawing Description Text (7):
FIG. 5 illustrate the ability of PEG-Ceramide to act as a bilayer stabilizing component. Multilamellar vesicles were prepared, as described in the examples, from DOPE:cholesterol:egg ceramide-PEG.sub.2000 at a ratio of A, 1:1:0.1 or B, 1:1:0.25. Other conditions were the same as for FIG. 2.

Drawing Description Text (21):
FIG. 19 illustrates the inhibition of fusion between DOPE:cholesterol:DODAC liposomes and anionic liposomes by PEG-ceramide. Liposomes were prepared from DOPE:cholesterol:DODAC, 40:45:15 (no PEG) or DOPE:cholesterol:DODAC:(C14:0) ceramide-PEG.sub.2000, 36:45:15:4 (4% PEG). Acceptor liposomes were prepared from DOPE:cholesterol:POPS, 25:45:30. A three-fold excess of acceptors was added to labelled vesicles after 30 s and the fluorescence monitored at 517 nm with excitation at 465 nm.

Drawing Description Text (23):
FIG. 21 illustrates the inhibition of fusion between DOPE:cholesterol:DODAC liposomes and erythrocyte ghosts by, PEG-ceramide. Liposomes were prepared from DOPE:cholesterol:DODAC:(C14:0) ceramide-PEG.sub.2000, 36:45:15:4 (4% PEG). Ghosts (50 .mu.M phospholipid) were added to donors (50 .mu.M total lipid) after 30 s and the fluorescence monitored at 517 nm with excitation at 465 nm.

Drawing Description Text (24):
FIGS. 22A-22F illustrate the fusion of fluorescent liposomes composed of DOPE:cholesterol:DODAC (40:45:15) or DOPE:cholesterol:DODAC:PEG-ceramide (35:45:15:5). LUVs composed of DOPE:cholesterol:DODAC (40:45:15) fused with RBCs (panels a and b); incorporation of PEG-ceramide (C8:0) into the LUVs at 5 mol % blocked fusion (panels c and d); however, when an exogenous sink for the PEG-ceramide was included, fusogenic activity was recovered within minutes (panels e and f). Panels a, c and e are views under phase contrast, and panels b, d and f are the same fields view under fluorescent light.

Detailed Description Text (22):
Cationic lipids have been used in the transfection of cells in vitro and in vivo (Wang, C-Y, Huang L., "pH sensitive immunoliposomes mediate target cell-specific delivery and controlled expression of a foreign gene in mouse," Proc. Natl. Acad. Sci. USA, 1987; 84:7851-7855 and Hyde, S. C., Gil, D. R., Higgins, C. F., et al., "Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy," Nature, 1993; 362:250-255). The efficiency of this transfection has often been less than desired, for various reasons. One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.

Detailed Description Text (23):

Cationic lipids useful in producing lipid based carriers for gene and oligonucleotide delivery include, but are not limited to, 3.beta.-(N-(N',N'-dimethylaminoethane)carbamoyl)cholesterol (DC-Chol); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE); diheptadecylamidoglycyl spermidine (DOGS); N-(1-(2,3dioleyloxy)propyl)-N-(2-(sperminecarboxamido)ethyl)-N,N-dimethyla mmonium trifluoroacetate (DOSPA); N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); N-(1-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-dioleyl-N,N-dimethylammonium chloride (DODAC); LIPOFECTIN, a commercially available cationic lipid comprising DOTMA and DOPE (GIBCO/BRL, Grand Island, N.Y.) (U.S. Pat. Nos. 4,897,355; 4,946,787; and 5,208,036 issued to Epstein, et at.); LIPOFECTACE or DDAB (dimethyldioctadecyl ammonium bromide) (U.S. Pat. No. 5,279,883 issued to Rose); LIPOFECTAMINE, a commercially available cationic lipid composed of DOSPA and DOPE (GIBCO/BRL, Grand Island, N.Y.); TRANSFECTAM, a commercially available cationic lipid comprising DOGS (Promega Corp., Madison, Wis.).

Detailed Description Text (70):
The spectra set forth in FIGS. 1-4 were all obtained using PEG conjugated to phosphatidylethanolamine through a carbamate linkage. In addition, however, the use of ceramide as an alternative anchor for the hydrophilic polymer was examined. PEG.sub.2000 was conjugated via a succinate linker to egg ceramide. FIG. 5 shows the .sup.31 P-NMR spectra obtained using mixtures of DOPE:cholesterol:egg ceramide-PEG.sub.2000 (1:1:0.1 and 1:1:0.25) over the temperature range of 0.degree. to 60.degree. C. At the lower molar ratio of PEG-ceramide, both bilayer and H.sub..PI. phase lipid are in evidence at most temperatures. However, at the higher PEG-ceramide molar ratio, the spectra are exclusively bilayer up to 60.degree. C. at which point a low field shoulder corresponding to H.sub..PI. phase lipid is visible. Unlike the spectra obtained using PEG-PEs, there was almost no isotropic signal when PEG-ceramide was used.

Detailed Description Text (90):
As an alternative bilayer stabilizing component, therefore, the ability of a neutral PEG-lipid species, i.e., PEG-ceramide, to inhibit fusion in this system was examined. PEG-ceramides have similar bilayer stabilizing properties to PEG-PEs. For these studies, PEG.sub.2000 was conjugated to ceramides of various fatty amide chain lengths through a succinate linker. Liposomes prepared from DOPE:DODAC:(C8:0) ceramide-PEG.sub.2000 (83:15:2) did not fuse in the presence of 300 mM NaCl. However, when an excess of POPC liposomes was added, fusion occurred fairly rapidly (FIG. 17). Similar results were observed when cholesterol was incorporated into the liposomes (DOPE:cholesterol:DODAC:(C8:0) ceramide-PEG.sub.2000, 38:45:15:2), although the rate of fusion was slower than with cholesterol-free liposomes (FIG. 17).

Detailed Description Text (96):
LUVs composed of DOPE:cholesterol:DODAC (40:45:15) fused rapidly and extensively with RBCs (FIG. 22, panels a and b). Prolonged incubation caused extensive lysis of the RBCs and numerous fluorescently labeled "ghosts" were formed. Incorporation of PEG-ceramide (C8:0) at 5 mol % blocked fusion (FIG. 22, panels c and d) and this effect was maintained for up to 24 hr. This effect was somewhat surprising since the (C8:0) ceramide can exchange rapidly (i.e., within minutes) between liposomal membranes. It appears that either the RBCs cannot act as a sink for the PEG-ceramide, or there were insufficient RBCs to remove enough PEG-ceramide to allow fusion. However, when an exogenous sink for the PEG-ceramide was included, fusogenic activity was recovered within minutes (FIG. 22, panels e and f).

Detailed Description Text (97):
When PEG-ceramides with longer fatty amide chains (i.e., C14:0 or C20:0) were used, there was little fusion over 24 hr, even in the presence of an exogenous sink. This again was surprising as substantial fusion is observed over this time frame in

liposomal systems when a sink is present. It was thought that some nonspecific interaction between the sink (POPC/cholesterol) and the RBCs was occurring which hindered the ability of the POPC:cholesterol liposomes to absorb the PEG-ceramide. To overcome this, the fusogenic liposomes were pre-incubated with the sink before adding RBCS. FIG. 23 shows the results obtained under these conditions using PEG-ceramide (C14:0). No fusion was observed after pre-incubation of the fusogenic LUVs with the sink for 5 minutes prior to addition of RBCs (FIG. 23, panels a and b). However, after a 1 hr pre-incubation, some fusion with RBCs was observed (FIG. 23, panels c and d), suggesting that under these conditions the PEG-ceramide could transfer out of the liposomes and became fusogenic. With longer incubations (2 hr), the pattern of fluorescent labeling changed. Rather than diffuse labeling of the RBC plasma membranes, extensive punctate fluorescence was observed (FIG. 23, panels e and f) and this pattern was maintained for up to 24 hr. The punctate fluorescence did not appear to be associated with cells and it may represent fusion of fluorescent liposomes with the sink, although previous fluorescent measurements of liposome-liposome fusion indicated that this did not occur to any appreciable extent. A second possibility is that exchange of the fluorescent probe over the longer time courses leads to labeling of the sink, although it seems unlikely that this would prevent fusion and labeling of the RBCS. When PEG-ceramide (C20:0) was used, there was no evidence for fusion after preincubation of LUVs with the sink for 5 min (FIG. 24, panels a and b), 1 hr (FIG. 24, panels c and d), 2 hr (FIG. 24, panels e and f), or for up to 24 hr (results not shown).

Detailed Description Text (98):
FIGS. 22-24 unequivocally establish that the liposomes of the present invention exhibit programmable fusion with intact cells. Firstly, liposomes composed of DOPE:cholesterol:DODAC (40:45:15) that contain no PEG-lipid fuse rapidly and extensively with RBCs. Secondly, when the liposomes contain 5 mol % PEG-lipid fusion is blocked regardless of the composition of the lipid anchor. Thirdly, in the presence of a sink to which the PEG-lipid can transfer, fusogenic activity can be restored at a rate that is dependent on the nature of the lipid anchor. Although exchange leading to fusion could not be demonstrated when the PEG-ceramide (C20:0) was used, it is believed this is a problem with the assay rather than a lack of fusogenic potential. In vivo there would be an almost infinite sink for PEG-lipid exchange.

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☐ 1. Document ID: US 6734171 B1

Using default format because multiple data bases are involved.

L3: Entry 1 of 15 File: USPT May 11, 2004

US-PAT-NO: 6734171

DOCUMENT-IDENTIFIER: US 6734171 B1

TITLE: Methods for encapsulating nucleic acids in lipid bilayers

DATE-ISSUED: May 11, 2004

INVENTOR-INFORMATION:

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US-CL-CURRENT: 514/44; 424/450, 435/320.1, 435/455, 435/458

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 2. Document ID: US 6673364 B1

L3: Entry 2 of 15 File: USPT Jan 6, 2004

US-PAT-NO: 6673364

DOCUMENT-IDENTIFIER: US 6673364 B1

TITLE: Liposome having an exchangeable component

DATE-ISSUED: January 6, 2004

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US-CL-CURRENT: 424/450; 428/402.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 3. Document ID: US 6586410 B1

L3: Entry 3 of 15 File: USPT Jul 1, 2003

US-PAT-NO: 6586410
DOCUMENT-IDENTIFIER: US 6586410 B1

TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

DATE-ISSUED: July 1, 2003

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US-CL-CURRENT: 514/44; 424/450, 435/6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 4. Document ID: US 6534484 B1

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DOCUMENT-IDENTIFIER: US 6534484 B1

TITLE: Methods for encapsulating plasmids in lipid bilayers

DATE-ISSUED: March 18, 2003

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US-CL-CURRENT: 514/44; 264/4.3, 264/4.6, 424/450, 436/829, 514/55, 514/851

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 5. Document ID: US 6465007 B1

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DOCUMENT-IDENTIFIER: US 6465007 B1

TITLE: Transgene expression in polarized cells

DATE-ISSUED: October 15, 2002

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US-CL-CURRENT: 424/450; 424/93.1, 424/93.2, 424/93.6, 435/320.1, 435/325, 435/455, 435/456, 514/2, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

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DOCUMENT-IDENTIFIER: US 6417326 B1

TITLE: Fusogenic liposomes

DATE-ISSUED: July 9, 2002

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Cullis; Pieter R.	Vancouver			CA
Choi; Lewis S. L.	Burnaby			CA
Monck; Myrna	Vancouver			CA
Bailey; Austin L.	Washington	DC		

US-CL-CURRENT: 530/324; 530/326, 530/327

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 7. Document ID: US 6287591 B1

L3: Entry 7 of 15 File: USPT Sep 11, 2001

US-PAT-NO: 6287591
DOCUMENT-IDENTIFIER: US 6287591 B1

TITLE: Charged therapeutic agents encapsulated in lipid particles containing four lipid components

DATE-ISSUED: September 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Semple; Sean C.	Vancouver			CA
Klimuk; Sandra K.	N. Vancouver			CA
Harasym; Troy	Vancouver			CA
Hope; Michael J.	Vancouver			CA
Ansell; Steven M.	Vancouver			CA
Cullis; Pieter	Vancouver			CA
Scherrer; Peter	Vancouver			CA
Debeyer; Dan	Vancouver			CA

US-CL-CURRENT: 424/450; 428/402.2, 435/177, 435/458, 514/44, 536/22.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 8. Document ID: US 6110745 A

L3: Entry 8 of 15 File: USPT Aug 29, 2000

US-PAT-NO: 6110745
DOCUMENT-IDENTIFIER: US 6110745 A

TITLE: Preparation of lipid-nucleic acid particles using a solvent extraction and direct hydration method

DATE-ISSUED: August 29, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zhang; Yuan-Peng	Mountain View	CA		
Scherrer; Peter	Vancouver			CA
Hope; Michael J.	Vancouver			CA

US-CL-CURRENT: 435/458; 264/4.1, 424/450, 424/93.2, 435/320.1, 435/325, 435/91.1, 435/91.31, 514/44, 536/23.1, 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 9. Document ID: US 5981501 A

L3: Entry 9 of 15 File: USPT Nov 9, 1999

US-PAT-NO: 5981501
DOCUMENT-IDENTIFIER: US 5981501 A

TITLE: Methods for encapsulating plasmids in lipid bilayers

DATE-ISSUED: November 9, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wheeler; Jeffery J.	Richmond			CA
Hope; Michael	Vancouver			CA
Cullis; Pieter R.	Vancouver			CA
Bally; Marcel B.	Bowen Island			CA

US-CL-CURRENT: 514/44; 264/4.3, 264/4.6, 424/450, 436/829, 514/55, 514/851

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 10. Document ID: US 5976567 A

L3: Entry 10 of 15 File: USPT Nov 2, 1999

US-PAT-NO: 5976567
DOCUMENT-IDENTIFIER: US 5976567 A

TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

DATE-ISSUED: November 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wheeler; Jeffery J.	Richmond			CA
Bally; Marcel B.	Bowen Island			CA
Zhang; Yuan-Peng	Vancouver			CA
Reimer; Dorothy L.	Vancouver			CA
Hope; Michael	Vancouver			CA
Cullis; Pieter R.	Vancouver			CA
Scherrer; Peter	Vancouver			CA

US-CL-CURRENT: 424/450; 435/458, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 11. Document ID: US 5965542 A

L3: Entry 11 of 15 File: USPT Oct 12, 1999

US-PAT-NO: 5965542
DOCUMENT-IDENTIFIER: US 5965542 A

TITLE: Use of temperature to control the size of cationic liposome/plasmid DNA complexes

DATE-ISSUED: October 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wasan; Ellen K.	Vancouver			CA
Bally; Marcel B.	Bowen Island			CA
Hope; Michael J.	Vancouver			CA
Reimer; Dorothy L.	Vancouver			CA
Ahkong; Quet Fah	Surry			CA

US-CL-CURRENT: 514/44; 424/450, 435/320.1, 435/458, 435/468, 435/91.1, 435/91.4, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 12. Document ID: US 5885613 A

L3: Entry 12 of 15 File: USPT Mar 23, 1999

US-PAT-NO: 5885613
DOCUMENT-IDENTIFIER: US 5885613 A
**** See image for Certificate of Correction ****

TITLE: Bilayer stabilizing components and their use in forming programmable fusogenic liposomes

DATE-ISSUED: March 23, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Holland; John W.	Glebe			AU
Madden; Thomas D.	Vancouver			CA
Cullis; Pieter R.	Vancouver			CA

US-CL-CURRENT: 424/450; 428/402.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 13. Document ID: US 5820873 A

L3: Entry 13 of 15 File: USPT Oct 13, 1998

US-PAT-NO: 5820873
DOCUMENT-IDENTIFIER: US 5820873 A
** See image for Certificate of Correction **

TITLE: Polyethylene glycol modified ceramide lipids and liposome uses thereof

DATE-ISSUED: October 13, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Choi; Lewis S. L.	Burnaby			CA
Madden; Thomas D.	Vancouver			CA
Webb; Murray S.	Vancouver			CA

US-CL-CURRENT: 424/283.1; 424/1.21, 424/184.1, 424/450, 424/812, 436/529, 436/535, 514/885

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 14. Document ID: US 5705385 A

L3: Entry 14 of 15 File: USPT Jan 6, 1998

US-PAT-NO: 5705385
DOCUMENT-IDENTIFIER: US 5705385 A

TITLE: Lipid-nucleic acid particles prepared via a hydrophobic lipid-nucleic acid complex intermediate and use for gene transfer

DATE-ISSUED: January 6, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bally; Marcel B.	Bowen Island			CA
Zhang; Yuan-Peng	Vancouver			CA
Reimer; Dorothy L.	Vancouver			CA
Wheeler; Jeffery J.	Richmond			CA

US-CL-CURRENT: 435/320.1; 264/4.1, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 15. Document ID: US 6734171 B1, WO 9918933 A2, AU 9913604 A, EP 1023048 A1

L3: Entry 15 of 15 File: DWPI May 11, 2004

DERWENT-ACC-NO: 1999-347142
DERWENT-WEEK: 200431

TITLE: Self-assembled lipid vehicle containing nucleic acid

INVENTOR: CULLIS, P R; KOJIC, L D ; LUDKOVSKI, O ; SARAVOLAC, E G ; SCHERRER, P ; WHEELER, J J ; ZHANG, Y

PRIORITY-DATA: 1997US-063473P (October 10, 1997), 1998US-0169573 (October 9, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 6734171 B1	May 11, 2004		000	A61K048/00
WO 9918933 A2	April 22, 1999	E	089	A61K009/127
AU 9913604 A	May 3, 1999		000	A61K009/127
EP 1023048 A1	August 2, 2000	E	000	A61K009/127

INT-CL (IPC): A61 K 9/127; A61 K 48/00; C12 N 15/88

Terms	Documents
L2 and transfect$	15

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☐ 1. Document ID: US 6652886 B2

Using default format because multiple data bases are involved.

L7: Entry 1 of 7 File: USPT Nov 25, 2003

US-PAT-NO: 6652886
DOCUMENT-IDENTIFIER: US 6652886 B2

TITLE: Biodegradable cationic copolymers of poly (alkylenimine) and poly (ethylene glycol) for the delivery of bioactive agents

DATE-ISSUED: November 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ahn; Cheol Hee	Salt Lake City	UT		
Kim; Sung Wan	Salt Lake City	UT		

US-CL-CURRENT: 424/501; 424/422, 424/423, 424/426, 424/484, 424/488, 424/489, 424/499, 514/772.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 2. Document ID: US 6616944 B2

L7: Entry 2 of 7 File: USPT Sep 9, 2003

US-PAT-NO: 6616944
DOCUMENT-IDENTIFIER: US 6616944 B2

TITLE: Self-assembling colloidal carriers for protein delivery

DATE-ISSUED: September 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kissel; Thomas	Staufen			DE
Breitenbach; Armin	Monheim			DE
Jung; Tobias	Aachen			DE
Kamm; Walter	Hofheim			DE

US-CL-CURRENT: 424/477; 424/409, 424/427, 424/434, 424/435, 424/457, 424/484, 424/491, 424/499, 514/2, 514/4, 524/801, 524/803, 525/437, 525/440, 525/56, 525/58, 525/63, 525/88, 525/89, 528/354, 528/361, 528/364

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 3. Document ID: US 6524613 B1

L7: Entry 3 of 7 File: USPT Feb 25, 2003

US-PAT-NO: 6524613
DOCUMENT-IDENTIFIER: US 6524613 B1

TITLE: Hepatocellular chimeraplasty

DATE-ISSUED: February 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Steer; Clifford J.	St. Paul	MN		
Kren; Betsy T.	Minneapolis	MN		
Bandyopadhyay; Paramita	Minneapolis	MN		
Roy-Chowdhury; Jayanta	New Rochelle	NY		

US-CL-CURRENT: 424/450; 514/44, 536/23.1, 536/24.1, 536/24.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 4. Document ID: US 6479292 B1

L7: Entry 4 of 7 File: USPT Nov 12, 2002

US-PAT-NO: 6479292
DOCUMENT-IDENTIFIER: US 6479292 B1

**** See image for Certificate of Correction ****

TITLE: Genetic alteration in plants using single-stranded oligodeoxynucleotide vectors

DATE-ISSUED: November 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Metz; Richard A.	Lawrenceville	NJ		
Frank; Bruce L.	Langhorne	PA		
Walther; Debra M.	Langhorne	PA		

US-CL-CURRENT: 435/468; 435/410, 435/469, 435/470, 514/44, 536/23.1, 536/26.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 5. Document ID: US 6271360 B1

L7: Entry 5 of 7 File: USPT Aug 7, 2001

US-PAT-NO: 6271360
DOCUMENT-IDENTIFIER: US 6271360 B1

TITLE: Single-stranded oligodeoxynucleotide mutational vectors

DATE-ISSUED: August 7, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Metz; Richard A.	Lawrenceville	NJ		
Frank; Bruce L.	Langhorne	PA		
Walther; Debra M.	Langhorne	PA		

US-CL-CURRENT: 536/23.1; 435/455, 435/458, 435/463, 435/468, 435/470, 435/471, 435/490, 536/26.6

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 6. Document ID: US 6013240 A

L7: Entry 6 of 7 File: USPT Jan 11, 2000

US-PAT-NO: 6013240
DOCUMENT-IDENTIFIER: US 6013240 A

TITLE: Nucleic acid containing composition, preparation and uses of same

DATE-ISSUED: January 11, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Behr; Jean-Paul	Strasbourg			FR
Demeneix; Barbara	Paris			FR
Lezoualch; Franck	Paris			FR
Mergny; Mojgan	Ivry Sur Seine			FR
Scherman; Daniel	Paris			FR
Boussif; Otmane	Strasbourg			FR

US-CL-CURRENT: 424/1.21; 435/6, 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 7. Document ID: US 5871713 A

L7: Entry 7 of 7 File: USPT Feb 16, 1999

US-PAT-NO: 5871713
DOCUMENT-IDENTIFIER: US 5871713 A

TITLE: Macromolecular polyamine iodine-containing compound, process for its preparation and its use as a contrast agent

DATE-ISSUED: February 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Meyer; Dominique	Saint-Maur			FR
Le Greneur; Soizic	Bures-sur-Yvette			FR

US-CL-CURRENT: 424/9.452; 424/9.451, 514/535, 514/563, 514/616, 525/911, 526/307, 528/363, 528/369, 560/37, 560/38, 560/43, 562/433, 562/455, 564/153